P2Y1 and P2Y12 receptor heterodimerisation: From recombinant systems to native detection

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Background and Purpose: Purinergic P2Y1 and P2Y12 receptors belong to the class A family of transmembrane G-protein coupled receptors (GPCRs) and have been demonstrated to exist as homodimers and oligomers and form heterodimers with other GPCRs. P2Y12 and protease-activated receptor 4 (PAR4) were recently reported to form a heterodimer with implications in receptor trafficking and signalling. Our unpublished studies suggest that hP2Y1 and hP2Y12 heterodimerise; therefore, the aim of this study was to investigate the functional relevance of receptor interaction, firstly in recombinant systems and then natively in microglial cells.

Experimental Approach: tSA201 cells, which are a transformed human kidney cell line, were transfected or co-transfected with hP2Y1 and hP2Y12 receptors tagged with HA (N-terminal) or a fluorescent protein (C-terminal). Their cellular co-localisation was determined using confocal microscopy and physical interaction by co-immunoprecipitation. Cell surface expression and receptor trafficking were quantified using an established anti-HA antibody surface ELISA approach. Native P2Y receptors were detected in mouse BV-2 microglial cells using RT- PCR and indirect immunofluorescence (using selective anti-P2Y1 and P2Y12 antibodies). A proximity ligation assay (PLA) was used to investigate native P2Y1 and P2Y12 receptor association.

Key Results: Previous co-immunoprecipitation experiments revealed that P2Y1 and P2Y12 receptors interact to form a heterodimer. This study shows that HA- and fluorescent protein tagged hP2Y1 and hP2Y12 receptors express predominantly at the cell surface in tSA201 cells. HA-hP2Y1 receptor expression was enhanced by 1.75 ± 0.311-fold (n = 3), at the cell surface when co-expressed with hP2Y12-eCFP but not vice versa. hP2Y1 and hP2Y12 internalisation to ADP (0–60 min, 10 μM) was maximum 30-min post-treatment (28.8% ± 6.2 and 20.8% ± 7.8, respectively). When receptors were co-expressed, ADP did not reduce receptor surface expression (108.4% ± 11.1 for hP2Y1 and 111.7% ± 10.8 for hP2Y12 30 min, relative to untreated; n = 3). Interestingly, native P2Y1 and P2Y12 receptors in BV-2 cells also appear to co-localise (Pearson's correlation coefficient = 0.684 ± 0.004 compared with P2Y1 (PCC = 0.095 ± 0.031) and P2Y12 (PCC = 0.162 ± 0.014) alone (n = 3, 200 cells); however, signals were detected intracellularly rather than at the cell surface. PLA is currently ongoing to assess if co-localisation translates to interaction.

Conclusions and Implications: hP2Y1 and hP2Y12 receptor heterodimersation impacted ADP-mediated internalisation when both receptors are overexpressed in tSA201 cells. Co-localisation studies in BV-2 cells suggest that the location of receptor interaction may differ depending upon the cell types explored. Further work is under way to investigate these differences.

Accepted author manuscript of the following conference abstract: Safar, M. A., Wood, R., Cunningham, M. R., & Kennedy, C. (2019). P2Y1 and P2Y12 receptor heterodimerisation: from recombinant systems to native detection. British Journal of Pharmacology, 176(16), 3049. https://doi.org/10.1111/bph.14681